Vestibular inputs to premotor respiratory interneurons in the feline C_1-C_2 spinal cord

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BS, University of Pittsburgh, 2003

Submitted to the Graduate Faculty of

The Department of Neuroscience in partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2005

UNIVERSITY OF PITTSBURGH

FACULTY OF ARTS AND SCIENCES

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The resting length of respiratory muscles must be altered during changes in posture in order to maintain stable ventilation. Prior studies showed that although the vestibular system contributes to these adjustments in respiratory muscle activity, the medullary respiratory groups receive little vestibular input. Additionally, previous transneuronal tracing studies and physiological experiments demonstrated that propriospinal interneurons in the C_1-C_2 spinal cord send projections to the diaphragm motor pool. The present study tested the hypothesis that C_1-C_2 interneurons mediate vestibular influences on diaphragm activity. Recordings were made from 145 C_1-C_2 neurons that could be antidromically activated from the C_5-C_6 ventral horn, 60 of which had spontaneous activity, during stimulation of vestibular receptors using electric current pulses or whole-body rotations in vertical planes. The firing of 19 of 31 spontaneously active neurons was modulated by vertical vestibular stimulation; the response vector orientations of most of these cells were closer to the pitch plane than the roll plane, and their response gains remained relatively constant across stimulus frequencies. Virtually all spontaneously active neurons responded robustly to electrical vestibular stimulation, and their response latencies were typically shorter than those for diaphragm motoneurons. Nonetheless, respiratory muscle responses to vestibular stimulation were still present after inactivation of the C_1-C_2 cord using large injections of either muscimol or ibotenic acid. These data suggest that C_1-C_2 propriospinal interneurons contribute to producing posturally-related responses of respiratory muscles, although additional pathways are also involved in generating these responses.

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1. Introduction

Multiple experimental approaches have established the role of the vestibular system in modulating respiratory muscle activity during changes in posture. Electrical stimulation of vestibular afferents has been shown to affect the firing of nerves innervating upper airway and respiratory pump muscles [1], with vestibular nerve stimulation producing alterations in phrenic nerve activity at latencies < 10 ms [2]. Another line of experimentation demonstrated that elimination of labyrinthine inputs resulted in increased spontaneous activity of both the diaphragm and abdominal musculature, as well as markedly diminished augmentations in abdominal muscle activity that ordinarily occur during nose-up tilts of an animal's torso [3]. Further, chemical stimulation in regions of the caudal vestibular nuclei produced prominent changes in the depth and frequency of breathing [4], and stimulation of other brain areas connected with portions of the vestibular nuclei that mediate vestibular influences on respiration, such as the rostral fastigial nucleus, anterior lobe (lobules I-V), and uvula of the cerebellum, affected respiratory muscle activity as well [5-10].

Knowledge regarding the neural pathways that convey vestibular signals to respiratory motoneurons is limited. Traditionally, the brainstem respiratory groups of the lateral reticular formation and caudal medulla were thought to contain the majority of premotor respiratory neurons [11-16]. However, recent experiments demonstrated that functional lesions of these cells failed to eliminate vestibulo-respiratory responses [1, 17, 18], and that vestibular stimulation could increase the activity of respiratory pump muscles without affecting the firing of respiratory group neurons [19, 20]. These data suggest that premotor neurons outside of the respiratory groups provide inputs to diaphragm and other respiratory motoneurons. Experiments

using the retrograde transneuronal tracer pseudorabies virus identified additional premotor respiratory neurons in the medial medullary reticular formation (MRF) and spinal cord [21-25]. Many MRF neurons receive inputs from the inner ear [26-28], and physiological studies confirmed that some cells in the MRF with projections to the phrenic motor pool have appropriate responses to vestibular stimulation to mediate labyrinthine influences on diaphragm motoneurons [29]. However, inactivation of the MRF using lidocaine or muscimol produced a large increase in spontaneous activity and responses to vestibular stimulation of respiratory muscles [30]. These findings suggest that additional premotor neurons must also be involved in relaying vestibular signals to diaphragm and abdominal motoneurons.

The remaining premotor respiratory neurons identified in transneuronal tracing studies were located in the spinal cord. Two distinct groups of spinal interneurons that make connections with diaphragm motoneurons were observed: propriospinal neurons located in the C_1-C_2 segments as well as segmental interneurons located in the ipsilateral intermediate zone of the C_5-C_6 gray matter [21-23, 25]. Propriospinal neurons located in the upper cervical spinal cord have been well characterized in the past. Some of these neurons were shown to receive inputs from respiratory group neurons [31-33] and to provide direct inputs to diaphragm motoneurons [34, 35], although they appear unessential for activation of respiratory muscles during respiration, emesis, and coughing [36]. Other reports have indicated that neurons located in similar regions of the upper cervical spinal gray matter respond to vestibular stimulation [37-41], raising the possibility that C_1-C_2 propriospinal neurons convey labyrinthine signals to respiratory motoneurons.

The goal of the present study was to examine the responses of C_1-C_2 neurons with axons that could be antidromically activated from the vicinity of phrenic motoneurons to whole-body

rotations in vertical planes that stimulate vestibular receptors, as well as to electrical stimulation of the vestibular nerve. A decerebrate cat model was employed in this study, as vestibulo-respiratory responses have been characterized extensively using this preparation [2, 18, 19, 30, 42, 43]. We tested the hypothesis that the majority of C_1-C_2 neurons with projections in the vicinity of the phrenic motor pool of the C_5-C_6 cord respond to vestibular stimulation with response dynamics similar to those for diaphragm motoneurons, thus suggesting that these cells are involved in producing vestibulo-respiratory responses during changes posture.

2. Methods and materials

All procedures used in this study were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee, and conformed to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

2.1. Surgical procedures

Experiments were conducted on 23 adult cats obtained from Liberty Research (North Rose, NY, USA). Each animal was initially anesthetized with isoflurane vaporized in oxygen. Administration of drugs was accomplished through catheters placed in both femoral veins, and blood pressure was monitored using a transducer (Millar Instruments, Houston, TX, USA) placed in a femoral artery. An infrared lamp and heating pad were used to maintain rectal temperature between 36 and 38 ˚C.

The animal was placed in a stereotaxic frame modified to pitch the animal's head down 30˚, in order to align the horizontal semicircular canals with the earth horizontal plane. The stereotaxic frame was mounted on a servo-controlled hydraulic tilt table (Neurokinetics,

Pittsburgh, PA, USA) capable of simultaneous rotations in the roll and pitch planes. Hip pins and a clamp on the dorsal process of the T_1 vertebra were used to secure the animal's body. A midcollicular decerebration was performed subsequent to ligation of the carotid arteries and aspiration of the cerebral cortex overlying the rostral brainstem. Dual laminectomies were performed to expose the C_1-C_2 and C_5-C_6 spinal cord. Anesthesia was stopped after decerebration and at least one hour prior to the onset of recording, and the animal was paralyzed using a10 mg/kg intravenous injection of gallamine triethiodide (Sigma, St. Louis, MO, USA). Paralysis was maintained using hourly supplemental injections of 5mg/kg of gallamine. During paralysis, animals were artificially respired using a positive-pressure ventilator, and end tidal CO_2 was maintained near 4%.

In 4 animals, diaphragm and abdominal muscle EMG responses elicited by electrical vestibular stimulation were recorded. To place EMG electrodes for recording diaphragm activity, a small incision was made through linea alba, and the liver and adjacent viscera were retracted to provide access to the costal portion of the muscle. On each side, a pair of Teflon-insulated stainless steel wires (Cooner Wire, Chatsworth, CA, USA) that was stripped of insulation for ~5 mm was sutured to the muscle epimysium together with an insulating patch of Silastic sheeting. Subsequently, the abdominal muscles were sutured closed, and wire pairs with uninsulated tips of ~ 1 mm were inserted into rectus abdominis and secured using sutures.

At the end of each recording session, animals were euthanized by a 120 mg/kg intravenous sodium pentobarbital injection, and the C_1-C_2 and C_5-C_6 spinal cord were removed and fixed in 10% formalin solution.

2.2. Data recording procedures

Extracellular recordings from C_1-C_2 neurons were made using epoxy-insulated tungsten microelectrodes with an impedance of 12 MΩ (A&M Systems, Sequim, WA, USA). Systematic tracking was done in 0.25 mm steps in the medial-lateral direction and 0.5 mm steps in the anterior-posterior direction from 1 - 2.25 mm lateral to the midline and from 0 – 5 mm caudal to the border of the brainstem with C_1. Current pulses were delivered to the C_5-C_6 ventral horn during tracking in order to stimulate the projections of C_1-C_2 propriospinal interneurons to the vicinity of diaphragm motoneurons. After isolating an antidromically-activated unit, the spontaneous activity of the cell and its responses to natural or electrical stimulation of vestibular afferents were determined.

Activity recorded by the microelectrode was amplified by a factor of 10,000 and filtered with a bandpass of 300 – 10,000 Hz. The output of the amplifier was fed into a window discriminator for the delineation of spikes from single units and sampled at 10,000 Hz using a 1401-plus data collection system (Cambridge Electronic Design, Cambridge, UK) and a Macintosh G4 computer (Apple Computer, Cupertino, CA, USA). Electrolytic lesions were made in two recording tracks per animal by passing a 20-μA negative current for 2 minutes, so that recording locations could be reconstructed.

EMG activity recorded from the diaphragm and rectus abdominis in 4 animals was amplified by a factor of 10,000, filtered with a band pass of 10–10,000 Hz, and full-wave rectified with a time constant of 10 msec. Subsequently, signals were sampled at 1,000 Hz using the Cambridge Electronic Design data collection system.

2.3. Antidromic activation of C_1-C_2 propriospinal neurons with projections to the vicinity of diaphragm motoneurons

In order to stimulate projections of C_1-C_2 propriospinal neurons to the vicinity of diaphragm motoneurons, a micromanipulator was used to lower a glass-insulated tungsten microelectrode with an impedance of approximately 0.5MΩ into the C_5-C_6 ventral horn. Multi-unit activity was recorded and fed into a Grass Instruments AM8 audio monitor (Quincy, MA, USA) while lowering the electrode. Electrode position was adjusted until discharges at the approximate frequency of respiration (~ 20/ min) were detected. Square-wave pulses, 0.15 ms in duration, were delivered at this location using a constant current stimulator. Once a C_1-C_2 neuron that responded to stimulation of the C_5-C_6 ventral horn was isolated, the stimulating electrode was moved dorsally and ventrally within the track, and often also into adjacent tracks, to determine the position where responses could be elicited at the lowest threshold. Responses were considered antidromic if they exhibited a sharp threshold, fixed latency, and collision block with spontaneous spikes. Neurons lacking spontaneous activity were verified as antidromic if responses could be consistently elicited at a fixed latency while delivering stimuli at high frequency (at least 100 Hz). Lesions were placed near stimulation sites by delivering a 20-μA negative current for 2 minutes, and the electrode was moved rostrally by ~1 mm prior to studying another unit.

2.4. Recording and analysis of responses to rotations in vertical planes

In 13 animals, we recorded the responses of C_1-C_2 neurons to stimulation of the vertical semicircular canals and otolith organs produced by tilting the entire animal about the pitch (transverse) and roll (longitudinal) axes. After verifying that its axon could be activated antidromically from the C_5-C_6 ventral horn, each cell was examined to ascertain if it responded to rotations in vertical planes. If a response was present, the plane of tilt that produced maximum

modulation of the cell's firing rate (response vector orientation) was determined. The "wobble" stimulus, a constant-amplitude tilt whose direction moves around the animal at a constant speed [44], was employed for this purpose. Wobble stimulation was typically delivered at 0.1 Hz, and sometimes also at 0.05, 0.2, or 0.5 Hz, at amplitudes up to 15˚. Clockwise wobble stimuli were generated by driving the pitch axis of the tilt table with a sine wave while simultaneously driving the roll axis with a cosine wave. Viewed from above during the stimulus, the animal's body appeared to wobble, having successive nose down, right ear down, nose up, and left ear down orientations. Inverting the signal to the pitch axis of the tilt table produced counterclockwise wobble stimuli. The phase differences between stimulus and response are reversed during the two directions of rotation [44]. Accordingly, the direction of the response vector orientation lies midway between the maximal response directions to clockwise and counterclockwise wobble stimulation.

To verify the response vector orientation for a given unit, we determined the cell's responses to sinusoidal tilts in a variety of fixed vertical planes, including the roll and pitch planes as well as planes oriented midway between roll and pitch, which were typically delivered at 0.1 Hz and at amplitudes of 7.5-10°. These fixed-plane tilts were generated by applying sine waves to the pitch axis, roll axis, or simultaneously to both axes of the tilt table. Driving both the roll and pitch axes at the same time produced stimuli in a plane between the two axes, the orientation of which was determined by the ratio of the signal sent to each axis. One side of the body was tilted down during the first half of the stimulus cycle, and the opposite side was tilted down during the second half of the stimulus cycle. After the response vector orientation was confirmed, fixed plane sinusoidal stimuli were subsequently delivered near this direction at

frequencies ranging from 0.02 to 0.5 Hz, in order to study the dynamics of responses to vestibular stimulation.

Neural activity recorded during rotations in vertical planes was binned (500 bins/cycle) and averaged over the sinusoidal stimulus period. A computer algorithm that employed a least-squares minimization technique was used to fit a sine wave to each response. Two parameters were used in characterizing the response sinusoid: phase shift from the stimulus sinusoid (phase) and amplitude relative to the stimulus sinusoid (gain). These measurements were corrected for the dynamics of the tilt table. Responses were considered significant if the signal-to-noise ratio (the amplitude of the sine wave to the root mean square amplitude of frequency components above the second harmonic) was ≥ 0.5 and only the first harmonic was prominent.

2.4.1. Recording and analysis of responses to electrical vestibular nerve stimulation

In 6 animals, we tested C_1-C_2 neurons that could be antidromically activated from the vicinity of the diaphragm motor pool for effects of electrical stimulation of the labyrinth. Previously described methods [2, 20, 45] were used to prepare the vestibular nerves for bipolar electrical stimulation. The tympanic bulla was exposed on both sides using a ventrolateral approach and opened to expose the promontory. The scala vestibuli was exposed, and a silver-silver chloride ball electrode insulated except at the tip was inserted in the direction of the vestibule. A second electrode was placed 1-2 mm away in the vicinity of the oval window. The electrodes were covered with warm paraffin wax and held in place using dental cement. Square wave current pulses, 0.2 ms in duration, were employed to stimulate the vestibular nerves. The effectiveness of vestibular nerve stimulation was determined by monitoring eye movements related to the vestibulo-ocular reflex (elicited by stimulation using a 50-shock train with a 3 msec interpulse

interval), and the difference in intensity required to produce eye and facial movements was noted. The facial movements were probably due to stimulus spread to motor axons in the facial nerve, the closest non-target nerve to the stimulating electrodes [46, 47]. During the recording session, the maximal stimulus intensity was limited to values lower than those shown to produce facial movements. Current intensities required to alter the activity of C_1-C_2 neurons were expressed as multiples of the threshold for producing field potentials recorded from the medial longitudinal fasciculus (MLF, which contains the medial vestibulospinal tract) in the rostral portion of the C_1 spinal segment. To determine whether a C_1-C_2 neuron received vestibular inputs, a 5- shock train with an interpulse interval of 3 ms at an intensity 5 times that required to produce MLF field potentials was employed to activate vestibular afferents. Stimulus trains were delivered at a rate of one per 1-2 s. Shorter trains were used to determine the minimal number of shocks required to produce a response. Response latencies were calculated from the first shock of the train as well as from the last shock of the shortest train that produced a response, referred to as the 'effective shock'. Additionally, the minimum stimulus intensity required to produce a response when using a 5-shock train was determined.

2.5. Effects of chemical inactivation of the C_1-C_2 spinal cord on vestibulo-respiratory responses.

In four animals, we determined the effect of bilateral inactivation of C_1-C_2 interneurons on EMG activity of the diaphragm and abdominal muscles that was elicited by electrical vestibular stimulation. To elicit EMG responses, electrical vestibular stimulation was delivered as described above, with the exception that trains of 25 or 50 shocks (interpulse interval of 3 msec) were applied every 1-3 s. We first confirmed that baseline responses were stable for at least one hour. Subsequently, a series of 0.1 µl saline injections containing 0.5 nmol muscimol (Sigma, St. Louis, MO, USA) were made in the C_1-C_2 spinal cord of 2 animals, and injections of 6 nmol

ibotenic acid (ICN Biomedicals, Aurora, OH, USA) were performed in 2 other cases. The solution was saturated with Fast Green dye so that the injection sites could be later confirmed histologically. Drugs were administered with the use of a 1 µl Hamilton syringe mounted on a micromanipulator, and multiple injections were made in several transverse planes of the C_1-C_2 cord, corresponding to the locations of electrode placement for neural recording in the previous experiments. Injection planes were 2 mm apart, with injections being placed 1and 3 mm lateral to the midline bilaterally, at depths of 1 and 3 mm within each track. Previous experiments showed that injections of this magnitude suppress neural activity within 1 mm of the injection site; thus, the injection paradigm that was employed should have suppressed neural activity throughout the C_1-C_2 gray matter [30, 36]. Averaged EMG responses to electrical vestibular stimulation were recorded subsequent to the drug infusion, and compared with pre-lesion activity. Infusion of large doses of muscimol or ibotenic acid into the spinal cord eventually resulted in a loss of spontaneous activity of respiratory muscles, presumably due to spread of the drugs into the brainstem respiratory groups, at which time animals were artificially ventilated as described above.

2.6. Tissue Analysis

In experiments where neuronal recordings were made, the C_1-C_2 and C_5-C_6 spinal cord were sectioned transversely at 100-µm and stained with thionine. Locations of recorded C_1-C_2 neurons were reconstructed using camera lucida relative to electrolytic lesion placement, positions of electrode tracks, and recorded microelectrode depth. Locations of stimulation sites within the C_5-C_6 grey matter were also reconstructed in a similar fashion. Stimulus spread from the lowest threshold stimulation site in the spinal gray matter was estimated at 10 µm per µA of current delivered [48], and a neuron was eliminated from our sample if the threshold current intensity to

elicit an antidromic response could have resulted in stimulus spread outside of the ventral gray matter.

The C_1-C_2 spinal cord was also sectioned transversely at 100-μm thickness subsequent to experiments involving muscimol or ibotenic acid injections, so that the location of dye marking the injection sites could be localized.

2.7. Data Analysis

Data were tabulated and analyzed with the use of Microsoft Excel software. Pooled data are presented as means ± one standard error.

3. Results

Recordings were made from 145 C_1-C_2 neurons that could be antidromically activated from the C_5-C_6 ventral horn using current intensities that did not produce stimulus spread beyond the ventral horn, assuming a current spread of 10 μm/μA [48]. The action potentials recorded from these units were biphasic or negative in polarity and over 1 ms in duration, suggesting that they originated from the soma. Sixty of the cells examined (41%) had spontaneous activity, with baseline firing rates ranging from 0.2 to 56 spikes/s (a mean of 6.5 ± 1.3 spikes/s), whereas the remaining 85 cells (59%) were silent. Antidromic thresholds ranged from 9-80 μA, with a mean of 32.6 ± 1 μA; Table 1 provides further details regarding the minimal current intensities required to elicit antidromic responses of spontaneously active neurons. The conduction velocities of C_1-C_2 neurons with projections to the C_5-C_6 ventral horn ranged from 8.4 to 80 m/s, with a mean of 33.6 ± 1.1 m/s.

3.1. Responses to Vertical Vestibular Stimulation

In 13 animals, we considered the effects of whole-body rotations in vertical planes on the activity of 63 C_1-C_2 neurons that could be antidromically activated from the C_5-C_6 ventral horn. Thirty-one of these cells were spontaneously active, and the remaining 32 were silent. Whereas no silent neurons were driven to fire by rotations in vertical planes, the activity of 19 of 31 spontaneously-active cells (61%) was modulated by natural vestibular stimulation at amplitudes $\leq 10°$. Table 1 summarizes the minimum current intensities delivered to the C_5-C_6 ventral gray matter required to produce antidromic activation of neurons that responded to vertical rotations. Examples of the responses of one C_1 propriospinal neuron to vertical vestibular stimulation are illustrated in Fig. 1. The response vector orientation (the plane of tilt producing the maximal response) for this neuron was between nose-down pitch and contralateral ear-down roll. The vertical vestibular response vector orientation was expressed using a head-centered coordinate system, with 0° corresponding to ipsilateral ear-down roll, 90° to nose-down pitch, 180° to contralateral ear-down roll, and –90° to nose-up pitch. In this coordinate system, the plane of the ipsilateral anterior semicircular canal is near 45°, the plane of the contralateral anterior semicircular canal is near 135°, and the planes of the ipsilateral and contralateral posterior semicircular canals are near –45° and –135°, respectively. Fig. 2 illustrates the response vector orientations, typically determined using wobble stimuli delivered at 0.1 Hz, for 16 of the 19 neurons that responded to rotations in vertical planes. The responses for the other three neurons were inconsistent from trial to trial or were present for only one direction of rotation (e.g., clockwise but not counterclockwise rotations), such that a response vector orientation could not be determined. Most (11 out of 16) of the C_1-C_2 neurons with projections to the C_5-C_6 ventral horn had response vector orientations closer to the pitch plane than to the roll plane. Fig. 2 also indicates the gains of the responses to wobble rotation at 0.1 Hz, which were calculated by

averaging the gain of neuronal activity elicited by clockwise and counterclockwise stimuli. The neurons with the largest amplitude responses to wobble stimulation were excited maximally by tilts in the nose-up direction.

Subsequently, rotations were performed at several frequencies (ranging from 0.02-0.5 Hz) in a plane oriented near that of the response vector orientation. Panel E of Fig. 1 is a Bode plot that shows the gain and phase of the responses of one neuron to tilts in the plane of the ipsilateral posterior semicircular canal and the contralateral anterior canal. Fig. 3 illustrates the dynamics of responses to planar tilts for all 16 units for which the response vector orientation could be determined. We could not always ascertain the effects of rotations at every target frequency, as units were often lost before the stimulus battery was complete. Nonetheless, it was clear that the gains of responses of most neurons were relatively similar across stimulus frequencies, and that on average the response phases were near stimulus position (see Fig. 3). These response characteristics are similar to those observed for respiratory muscles during rotations in vertical planes [3, 42].

3.2. Responses to Electrical Vestibular Stimulation

In 6 other animals, we ascertained whether delivery of current pulses to the vestibular nerve on either side altered the activity of C_1-C_2 neurons with projections to the C_5-C_6 ventral horn. Responses were recorded from 82 units during electrical stimulation of vestibular afferents, 29 of which were spontaneously active and 53 of which were silent. Twenty-seven of the 53 silent neurons (51%) responded to electrical vestibular stimulation on at least one side, as well as 28 of the 29 spontaneously active neurons (97%). Furthermore, the firing of 26 of the 29 spontaneously active units (90%) was altered by stimulation of either vestibular nerve, whereas 2 cells responded to stimulation of one side but not the other. The changes in activity recorded

from spontaneously active neurons typically were complex, and consisted of both increases and decreases in firing relative to baseline; stimulation of the ipsilateral or contralateral nerve usually produced similar changes in neuronal discharges. Fig. 4 shows examples of the responses to stimulation of the ipsilateral vestibular nerve of a C_1 neuron having an axon that could be activated antidromically from the C_6 ventral horn. Table 1 indicates the minimum current intensities delivered to the C_5-C_6 ventral gray matter that were required to produce antidromic activation of all spontaneously active neurons having firing that was altered by vestibular nerve stimulation.

By definition, only excitatory responses could be detected for silent neurons, as inhibition of the cells could not be perceived. For this reason, we did not ascertain the latencies or other parameters of responses to vestibular stimulation of silent units. Alterations in the firing of 26 of the 28 spontaneously active neurons that responded to vestibular nerve stimulation were sufficiently sharp to accurately determine the response latency. For all but 3 of the units, only a single shock delivered to the vestibular nerve was needed to evoke a change in firing. The response latency after stimulation of the ipsilateral vestibular nerve was 10.7 ± 1.8 ms from the first shock of the stimulus train, and 9.4 ± 1.3 ms from the effective shock (the last stimulus pulse of the shortest train that evoked an alteration in firing). Similarly, the latencies of responses to contralateral vestibular nerve stimulation were 9.5 ± 1.4 ms from the first pulse of the stimulus train, and 8.2 ± 1.2 ms from the effective shock. A Mann-Whitney test revealed no differences in the latencies of responses to ipsilateral and contralateral vestibular nerve stimulation (2-tailed P value was 0.99 for the response latency measured from the initial shock and 0.73 for the response latency determined from the effective pulse). Fig. 5 indicates the latencies of responses of each neuron to vestibular nerve stimulation. These latencies were

measured from the effective shock, and reflect the earliest response to stimulation of the ipsilateral or contralateral side. The latencies of responses of 21 of the 26 neurons (81%) were shorter than the average response latency to electrical vestibular stimulation previously determined for diaphragm motoneurons: 9.1 ± 3.2 ms [2].

During stimulation trials, we used current intensities that were subthreshold for activating efferents in the facial nerve, which was the nearest non-target nerve to the electrodes in the labyrinth [46]. The average threshold for activating vestibular nucleus neurons using a single shock, as estimated by the presence of field potentials recorded from the medial vestibulospinal tract in the MLF of the C_1 spinal segment, was 58 ± 10 μA for the left side and 40 ± 3 μA for the right side. In contrast, the mean threshold for producing facial movements using a train of 50 pulses was 453 ± 76 μA when stimulating the left labyrinth and 537 ± 50 μA for the right labyrinth. Because we always stimulated the labyrinth using stimulus intensities ≤ 5 times the threshold for activating vestibular nucleus neurons, it seems likely that changes in neuronal firing elicited by this delivery of current were the result of activation of vestibular afferents, and not other afferent fibers.

3.3. Locations of recording sites

The locations of C_1-C_2 neurons that could be antidromically activated from the C_5-C_6 ventral horn were distributed from 0.5 to 2.0 mm lateral to the midline and 1 to 3.5 mm deep from the surface of the cord. The majority of units was located in the ventral horn (Rexed's laminae VII or VIII), although some cells were recorded in lamina IV-VI. Silent and spontaneously active neurons, as well as cells having and lacking responses to natural or electrical stimulation, were intermingled in the same regions, and did not appear to be localized into specific areas of the cord.

3.4. Effects of inactivation of C_1-C_2 neurons on vestibulo-respiratory responses

In the remaining 4 animals, we evaluated the effects of chemical inactivation of the C_1-C_2 segments on the responses of the diaphragm and abdominal muscles to electrical vestibular stimulation. The muscimol or ibotenic acid solution delivered to the spinal cord was saturated with Fast Green dye, so that the spread of the solution through the tissue could be evaluated histologically. Infusion of muscimol into the C_1-C_2 spinal cord resulted in a marked attenuation of both diaphragm and abdominal muscle responses to vestibular stimulation, although responses were clearly present 1 hour subsequent to injections, when the experiment was terminated. Similar results were observed in 2 animals injected with ibotenic acid. Fig. 6 shows an example of diaphragm activity recorded before and at several time points subsequent to ibotenic acid injections in one animal. The attenuation of responses to vestibular stimulation was accompanied by a loss of spontaneous firing of the respiratory muscles, presumably because of spread of the drugs into the brainstem respiratory groups. Completeness of the lesions was subsequently evaluated by observing the location of Fast Green dye in histological sections. In all experiments, the dye completely filled the C_1-C_2 spinal segments, and had clearly infiltrated into the caudal medulla and the C_3 and C_4 segments. These results suggest that the C_1 and C_2 neurons are not essential for relaying vestibular signals to respiratory motoneurons. The basis of the eventual decrement of vestibulo-respiratory responses following the drug injections is not certain. It seems likely that these effects were related to the deterioration of the animal's condition and reduction of the excitability of respiratory motoneurons as the agents diffused away from the injection site, as opposed to specific effects on the C_1-C_2 respiratory premotor neurons.

4. Discussion

Previous anatomical [25] and physiological [34, 35] studies revealed that a population of propriospinal neurons in the C_1 and C_2 spinal cord make synaptic connections with diaphragm motoneurons. The present study showed that a preponderance of C_1-C_2 neurons having axonal branches that course in the vicinity of diaphragm motoneurons in the C_5-C_6 ventral horn respond at short latency to stimulation of the vestibular nerve. Furthermore, the spatial and temporal properties of the responses of a majority of these neurons to vertical vestibular stimulation resemble those of respiratory muscles [42]. These findings suggest that C_1-C_2 propriospinal neurons participate in relaying labyrinthine signals to diaphragm motoneurons, and are thus components of the neural circuitry that mediates vestibulo-respiratory responses.

Although we demonstrated that axonal branches of the C_1-C_2 neurons that were studied coursed through the C_5-C_6 ventral gray matter, based on a conservative estimate of current spread from the tip of our stimulating electrode [48], we did not directly demonstrate that these axons made synaptic connections with diaphragm motoneurons. Nonetheless, since a large population of premotor inspiratory neurons is known to exist in the C_1-C_2 spinal segments [32, 34, 35, 49], and is in fact the only group of propriospinal neurons that has been described in these segments with projections to the C_5-C_6 gray matter, it seems likely that many of the cells included in our sample did relay signals to the diaphragm motor pool. Prior physiological studies revealed that many C_1-C_2 propriospinal neurons exhibit inspiratory-related firing [2, 6, 7], whereas most of the neurons we recorded were silent or did not overtly appear to have firing at the frequency of respiration. This may be due to the fact that we maintained a relatively low end-tidal CO_2 (no more than 4%) in the animals in order to obtain robust responses to vestibular

stimulation. As a result, respiratory rhythmogenesis by the central nervous system was likely suppressed.

Further evidence that C_1-C_2 respiratory neurons mediate vestibulo-respiratory responses comes from the prevalence of vestibular inputs to these cells. Virtually all of the spontaneously active cells that were antidromically activated from the C_5-C_6 gray matter responded to electrical stimulation of the vestibular nerve, and 19 of 32 spontaneously firing cells responded to rotations in vertical planes at modest amplitudes ($\leq 10°$). It is certainly possible that the activity of a much larger fraction of these cells would have been modulated by natural vestibular stimulation if larger stimulus amplitudes could have been employed and if all directions of motion (including horizontal rotation and vertical heave) could have been included in the stimulus battery. Thus, even if only a subset of the propriospinal neurons that were examined made connections with diaphragm motoneurons, it is still likely that these cells received vestibular inputs.

The latency of responses to vestibular stimulation of the majority of C_1-C_2 propriospinal neurons with axons that were activated antidromically from the C_5-C_6 ventral horn was shorter than that previously noted for diaphragm motoneurons. Considering that these propriospinal neurons also had rapid conduction velocities (mean of 33.6 ± 1.1 m/sec), they certainly could contribute to producing the onset of diaphragm responses to vestibular stimulation. It is possible that C_1-C_2 cells activated at longer latency contribute to producing the later components of vestibulo-respiratory responses, which persist for over 40 msec even after a short train of 5 stimuli was applied to the vestibular nerve [2].

Respiratory motoneurons and muscles respond most powerfully to rotations in vertical planes, and their activity normally increases during nose-up rotations [3, 19, 42]. Accordingly, 11 out of 16 C_1-C_2 propriospinal neurons were observed to have response vector orientations that

were nearer to the pitch plane than to the roll plane. Furthermore, the neurons exhibiting the largest response gains had response vector orientations near nose-up pitch. On average, the response gains of C_1-C_2 propriospinal neurons were consistent across stimulus frequencies, and the response phase at each frequency was near stimulus position. These response dynamics also resemble those of respiratory muscles [3, 19, 42]. However, the physiological role of C_1-C_2 premotor respiratory neurons with response vector orientations in the roll plane or near nose-down pitch remains to be determined. One possibility is that many of the neurons activated by nose-down pitch were inhibitory, and thus contributed to increasing diaphragm activity during nose-up pitch through disinhibition. Another possibility is that the neurons activated by roll rotations are normally silent in conscious animals, and only are recruited during specialized movements requiring the stiffening of the diaphragm during ear down-rotations. These possibilities await further investigation.

Despite the fact that C_1-C_2 propriospinal neurons are likely to play a role in producing vestibulo-respiratory responses, extremely large injections of the excitotoxin ibotenic acid or the GABA agonist muscimol into the C_1-C_2 spinal cord did not abolish these responses. It seems likely that the preservation of these responses was not due to a partial inactivation of neurons in the C_1-C_2 gray matter. Previous literature indicates that the drug doses used should have completely affected all cell bodies in the target region [30, 36]. In addition, the Fast Green dye dissolved in the injection solution completely filled the spinal gray matter of C_1-C_2, and also infiltrated into the caudal medulla and the C_3-C_4 spinal segments. These drug doses additionally produced large fluctuations in blood pressure and abolished tonic respiratory activity, which were likely due to diffusion of the agents to sites at some distance from the target region.

These findings suggest that multiple populations of neurons are likely to participate in producing vestibulo-respiratory responses. Although neurons in the medullary respiratory groups are not essential for producing vestibulo-respiratory responses [1, 18-20], some cells in the ventral respiratory group do receive labyrinthine signals and may contribute to these responses [17, 19]. Neurons in the medial medullary reticular formation also may contribute to producing the inhibitory components of vestibulo-respiratory responses [29, 30]. Anatomical studies using the transneuronal transport of pseudorabies virus have demonstrated that a large number of segmental interneurons make synaptic connections with respiratory motoneurons [25], although further experiments will be required to ascertain whether these cells contribute to producing vestibulo-respiratory responses.

In summary, the present data suggest that a subpopulation of C_1-C_2 neurons receiving vestibular inputs and having projections to the diaphragm motor pool participate in generating vestibulo-respiratory responses, although these cells are not essential for producing the responses. It seems likely that labyrinthine signals are conveyed to diaphragm motoneurons through several parallel pathways, which include projections from the ventral respiratory group, medial medullary reticular formation, C_1-C_2 propriospinal neurons, and segmental respiratory interneurons. An important next step in this research will be to define the physiological role of each of these regions in adjusting respiratory muscle activity during movement and postural alterations.

Table 1

Thresholds for eliciting antidromic responses of spontaneously active C_1-C_2 neurons from the C_5-C_6 ventral horn. The presence or absence of neuronal responses to rotating the animal's body in vertical planes (natural stimulation) or electrically stimulating the vestibular nerve (electrical stimulation) is also indicated.

Antidromic Threshold (μA)	**Response to Natural Stimulation**		**Response to Electrical Stimulation**	
	Present	***Absent***	***Present***	***Absent***
0-10	0	0	1	0
11-20	1	0	3	0
21-30	5	3	9	0
31-40	9	4	11	0
41-50	3	4	2	1
51-60	1	0	2	0
61-70	0	0	0	0
71-80	0	1	0	0
Total	19	12	28	1

Figure 1

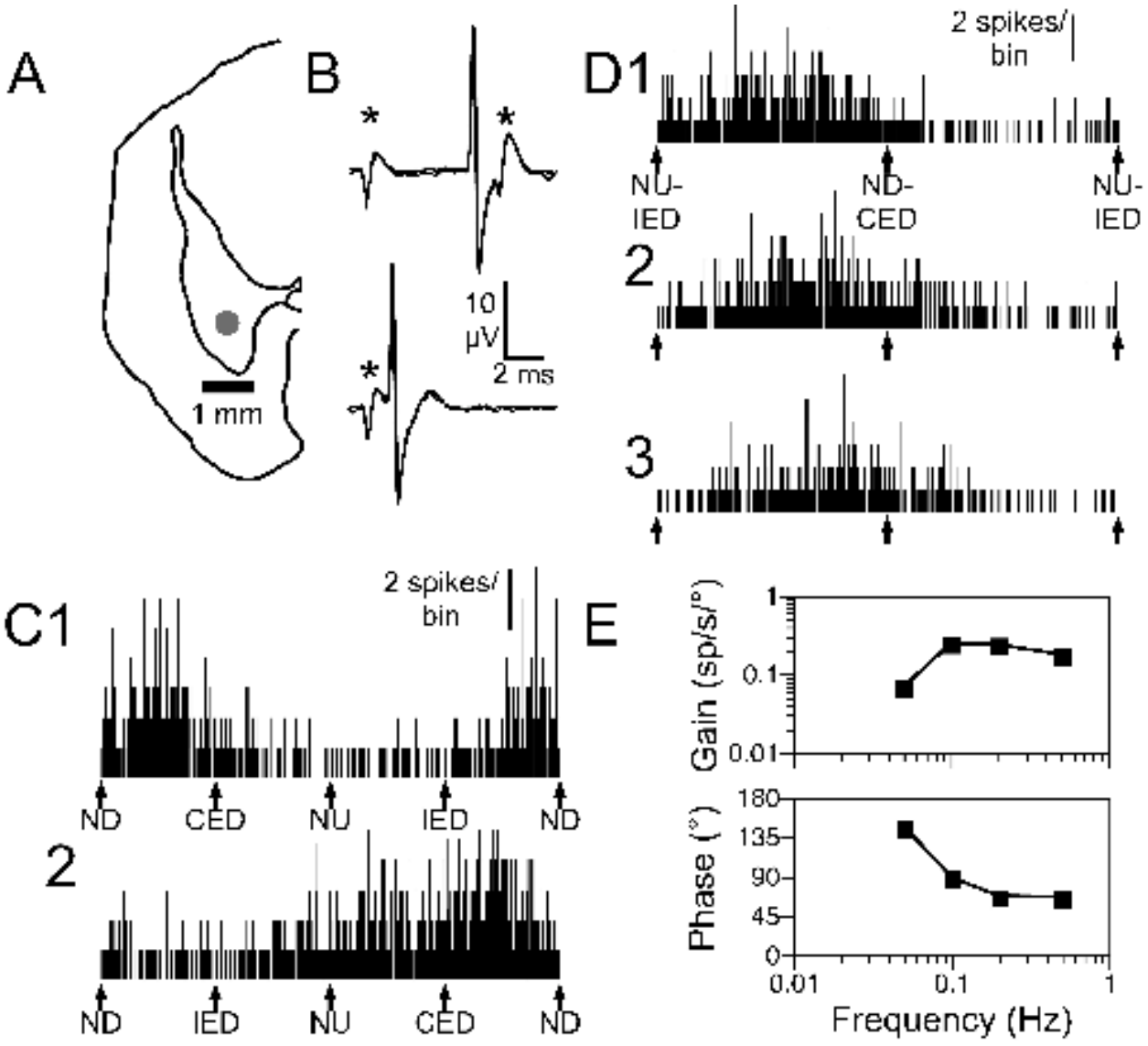

A
1 mm
B
10 μV
2 ms
D1
2 spikes/ bin
NU-IED
ND-CED
NU-IED
2
3
C1
2 spikes/ bin
ND
CED
NU
IED
ND
2
ND
IED
NU
CED
ND
E
Gain (sp/s/°)
1
0.1
0.01
Phase (°)
180
135
90
45
0
0.01
0.1
1
Frequency (Hz)

Figure 2

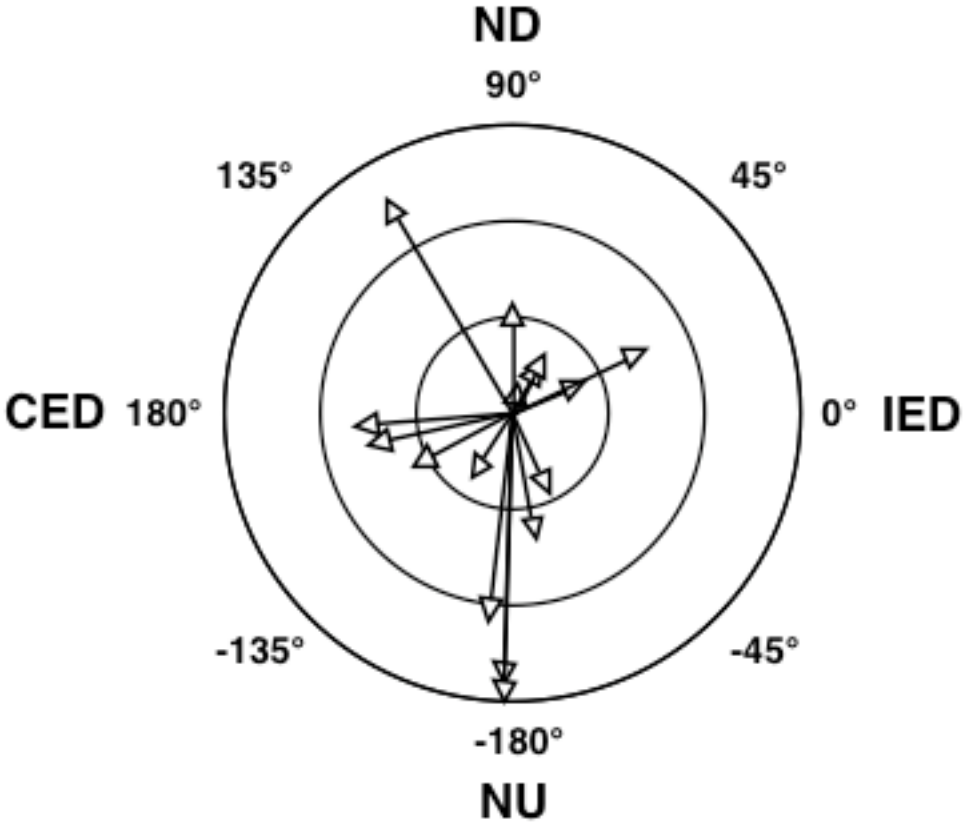


Figure 3

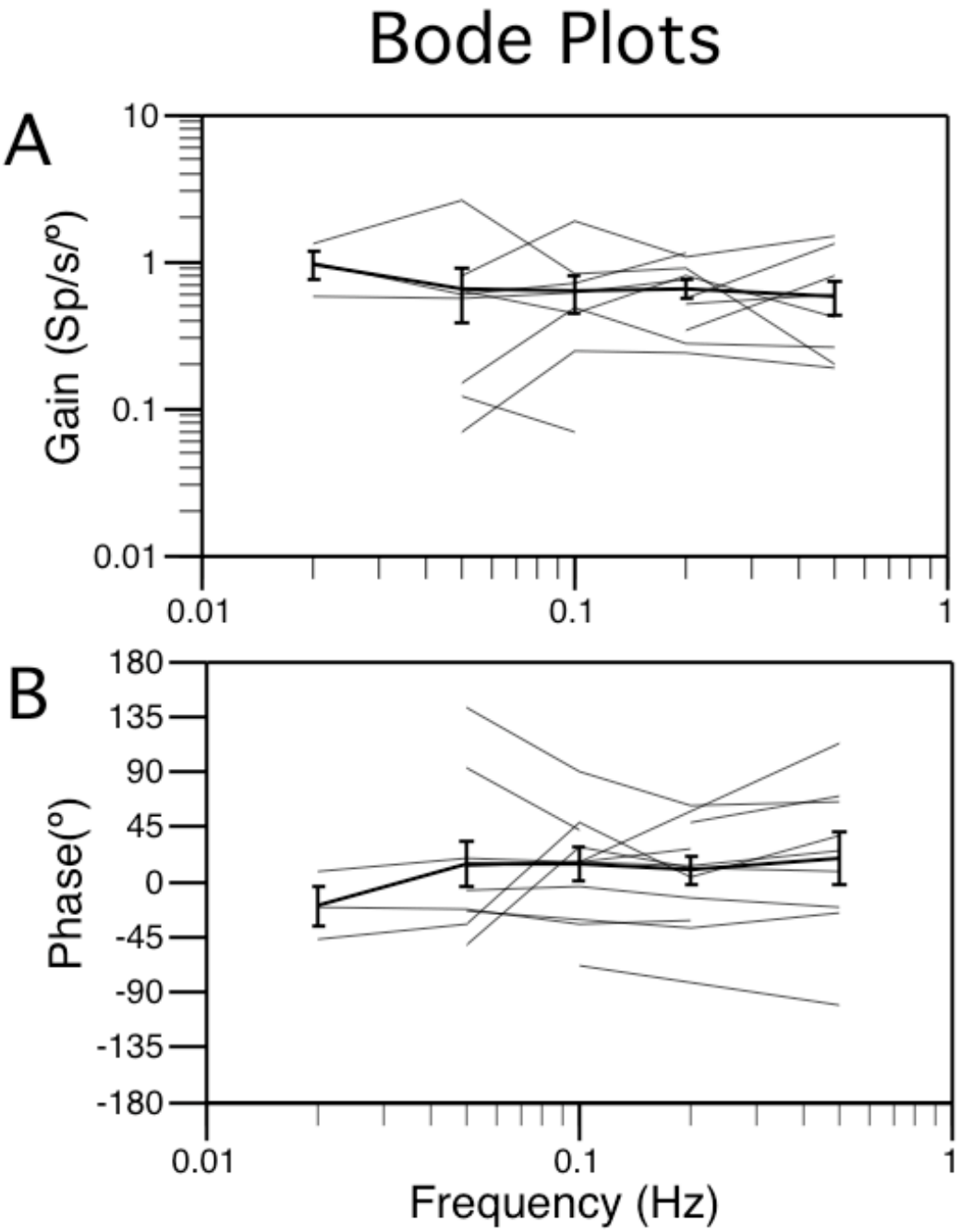

Bode Plots
A
Gain (Sp/s/°)
10
1
0.1
0.01
0.01
0.1
1
B
Phase(°)
180
135
90
45
0
-45
-90
-135
-180
0.01
0.1
1
Frequency (Hz)

Figure 4

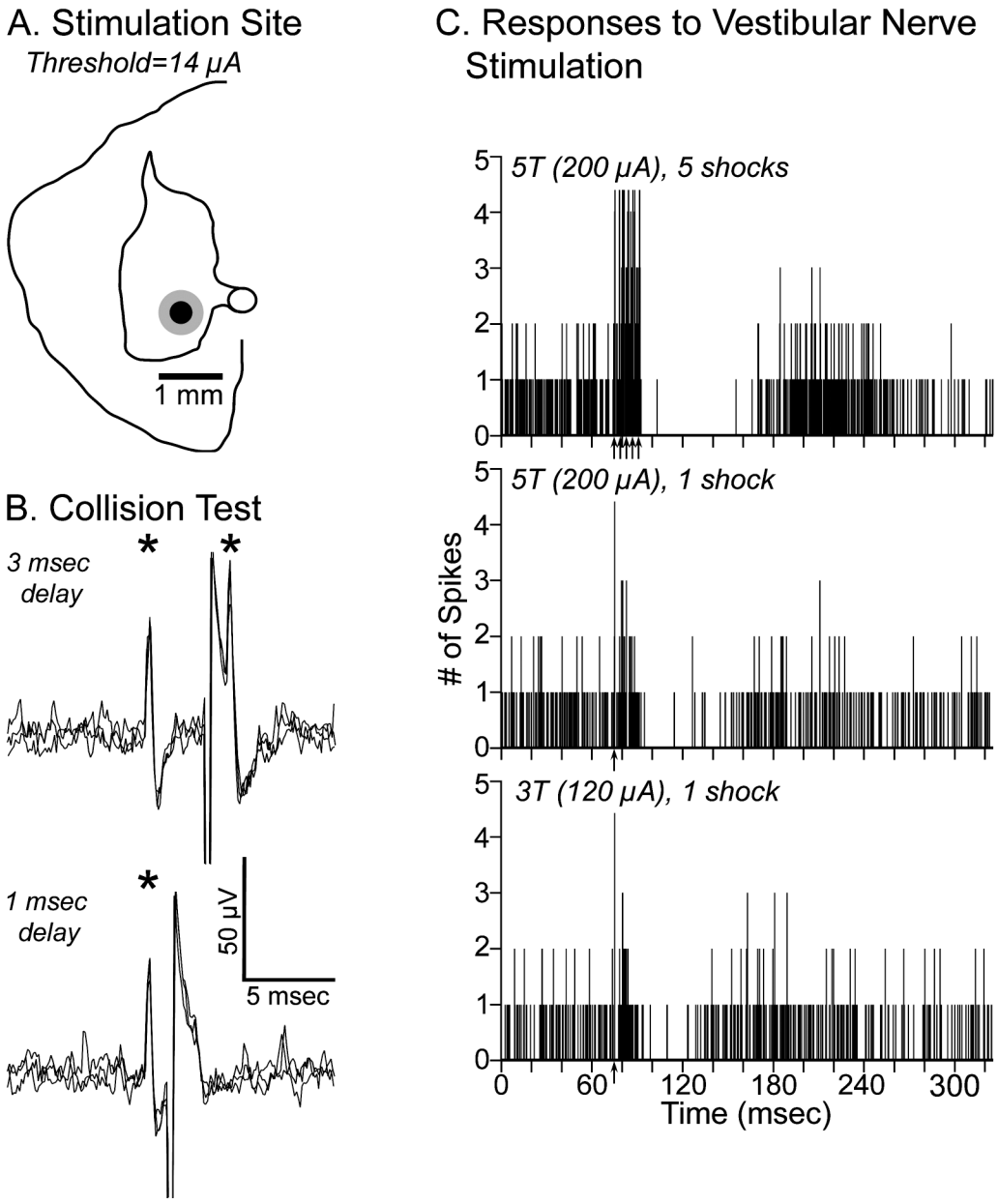

A. Stimulation Site
Threshold=14 μA
1 mm
B. Collision Test
3 msec delay
1 msec delay
50 μV
5 msec
C. Responses to Vestibular Nerve Stimulation
5T (200 μA), 5 shocks
5T (200 μA), 1 shock
3T (120 μA), 1 shock
# of Spikes
Time (msec)
0
60
120
180
240
300

Figure 5

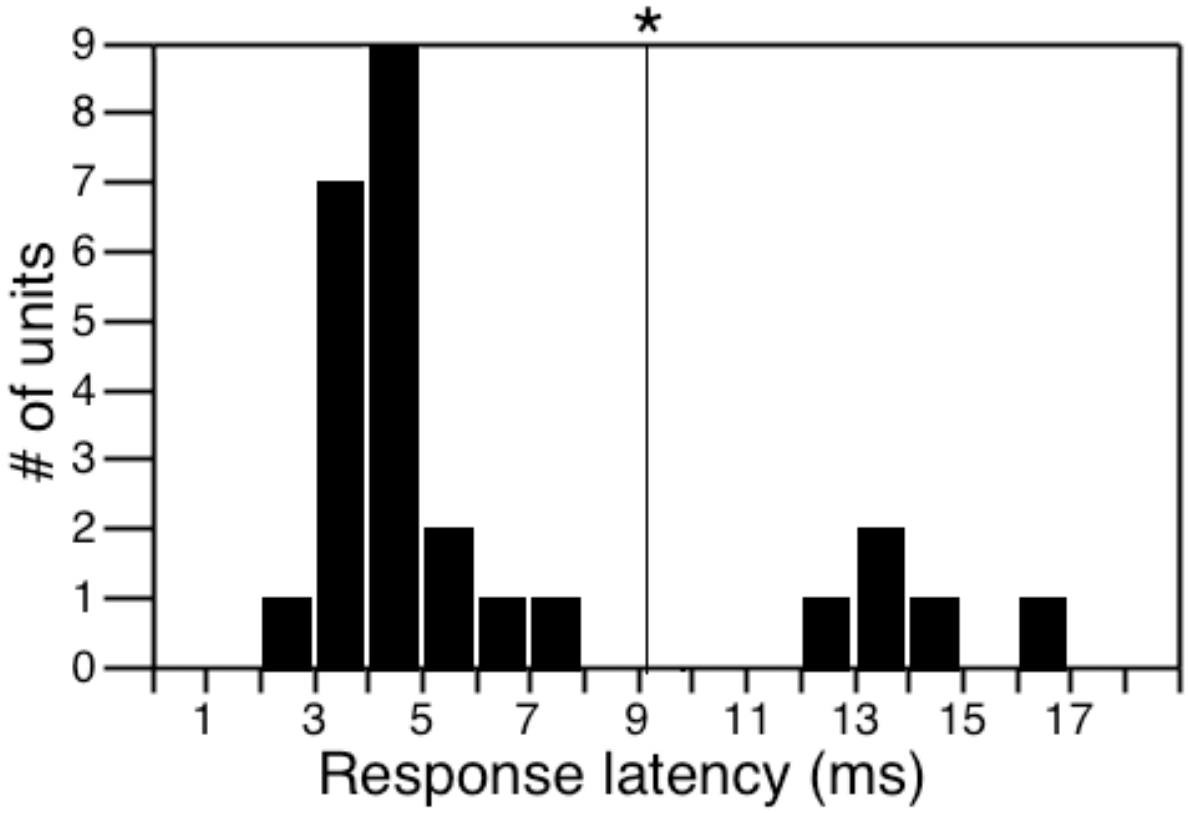
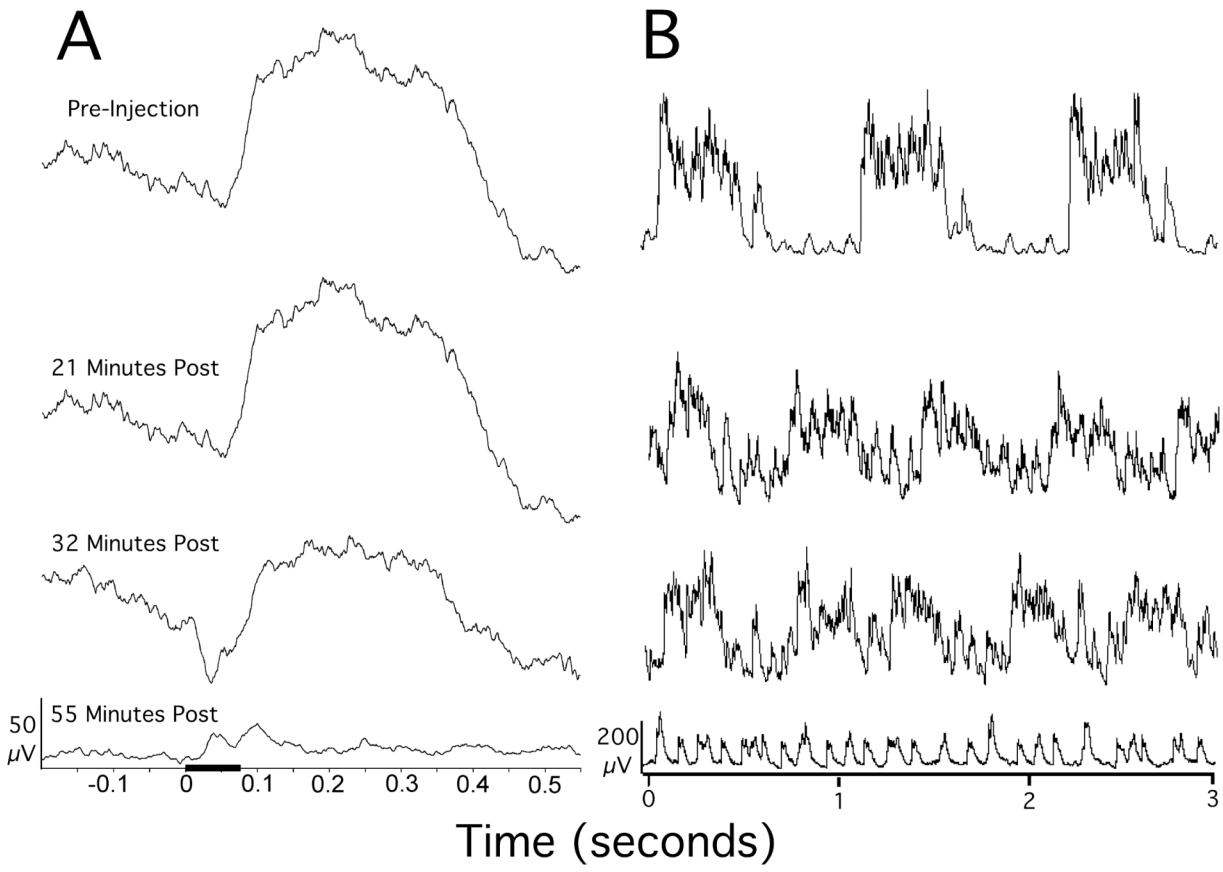


Figure 6


A
B
Pre-Injection
21 Minutes Post
32 Minutes Post
55 Minutes Post
50
μV
-0.1
0
0.1
0.2
0.3
0.4
0.5
200
μV
0
1
2
3
Time (seconds)

APPENDIX A

Figure Ledgends

Figure 1. Responses of a C_1 propriospinal neuron with a projection to the C_5 ventral horn to vertical vestibular stimulation. **A:** Antidromic stimulation site in the C_5, along with an indication of the area of tissue affected by the threshold current intensity (16 μA), assuming a likely stimulus spread of 10 μm/μA (shaded area). **B:** Collision test verifying that responses to C_5 stimulation were antidromic; action potentials are designated by asterisks. When the interval between a spontaneously occurring action potential and the stimulus was reduced from 5 to 1 ms, collision block was observed. Three sweeps were superimposed to produce each trace. **C:** Averaged neuronal responses to ~150 repetitions of wobble stimuli, constant amplitude tilts whose direction moves around the animal at constant speed, which were delivered at a frequency of 0.5 Hz and an amplitude of 7.5°. Trace C1 is the response to clockwise rotation, whereas C2 is the response to counterclockwise rotation. **D:** Averaged responses to 10° sinusoidal tilts delivered in a plane near the response vector orientation. Trace D1 is the averaged response to 25 tilts delivered at 0.1 Hz, whereas D2 is the averaged response to 50 tilts delivered at 0.2 Hz and D3 is the averaged response to 150 tilts presented at 0.5 Hz. **E:** Bode plot indicating the gain and phase (relative to stimulus position) of responses to tilts in the plane of response vector orientation. Abbreviations: CED, contralateral ear down roll; IED, ipsilateral ear down roll; ND, nose down pitch; NU, nose up pitch; Sp/s/°, spikes per second per degree of stimulation.

Figure 2. Response vector orientations of 16 spontaneously active cells to vertical vestibular stimulation. These orientations were determined using wobble stimuli, typically delivered at 0.1 Hz. The position of each arrow around the circumference of the circular plot reflects the best direction of tilt for exciting a neuron. The response gain in spikes per second per degree (sp/s/˚) of rotation is indicated by the length of each arrow, with the full radius of the circle set as the amplitude of the largest response, 1.55 sp/s/˚. Abbreviations: CED, contralateral ear down roll tilt; IED, ipsilateral ear down tilt; ND, nose down tilt; NU, nose up tilt.

Figure 3. Bode plots indicating the gain and phase (relative to stimulus position) of responses to tilts in the plane of response vector orientation, across a frequency range of 0.02 to 0.5 Hz. The bold lines represent the averaged response at each frequency tested; error bars indicate one SEM. Some cells were not tested across all frequencies, typically because they were lost before the stimulus battery was completed. Abbreviation: Sp/s/˚, spikes per second per degree of stimulation.

Figure 4. Responses of a C_1 neuron with a projection to the C_6 ventral horn to electrical stimulation of vestibular afferents. **A:** Antidromic stimulation site in C_6, along with an indication of the area of tissue affected by the threshold stimulus intensity of 14 μA, assuming a current spread of 10 μm/μA. **B:** Collision test verifying that responses to C_6 stimulation were antidromic; action potentials are designated by asterisks. Three sweeps were superimposed to produce each trace. **C:** Poststimulus histograms (~ 60 sweeps binned/trace) showing the responses of this neuron to stimulation of the contralateral vestibular nerve. Trace C1 was elicited by a train of 5 shocks with a 3 ms interpulse interval delivered at 200 μA, which was 5 times the threshold (T) for eliciting field potentials recordable from vestibular nucleus neurons.

Trace C2 was elicited by a single shock at 5T intensity, whereas trace 3 is the response to a single shock of 120 μA intensity (3T). Arrows designate when stimuli were delivered.

Figure 5. Onset latencies from the effective shock of neuronal responses to vestibular nerve stimulation. The latencies presented represent those elicited by stimulation of the vestibular nerve (either ipsilateral or contralateral to the recording site) that provided the shortest latency change in neuronal firing. The vertical line and asterisk indicate the mean response latency of diaphragm motoneurons to vestibular nerve stimulation, 9.1±3.2 (SEM) ms [2]. A majority of neurons (21/26) responded to vestibular stimulation at shorter latencies than did diaphragm motoneurons.

Figure 6. Diaphragm EMG responses to electrical vestibular nerve stimulation before and after ibotenic acid infusion into the C_1-C_2 spinal cord. **A:** Averaged diaphragm EMG activity (to approximately 150 stimulus presentations) elicited by a train of 25 shocks of 600 μA intensity delivered to the ipsilateral vestibular nerve; the duration of vestibular nerve stimulation is indicated by a thick bar. The times at which responses were recorded relative to the completion of ibotenic acid infusion into the spinal cord are indicated above each trace. **B:** Spontaneous diaphragm activity at each time point for which responses to vestibular stimulation were recorded.

BIBLIOGRAPHY

1. Shiba, K., M.S. Siniaia, and A.D. Miller, Role of ventral respiratory group bulbospinal expiratory neurons in vestibular-respiratory reflexes. J Neurophysiol, 1996. 76(4): p. 2271-2279.

2. Yates, B.J., J. Jakus, and A.D. Miller, Vestibular effects on respiratory outflow in the decerebrate cat. Brain Res, 1993. 629(2): p. 209-217.

3. Cotter, L.A., et al., Effects of postural changes and vestibular lesions on diaphragm and rectus abdominis activity in awake cats. J Appl Physiol, 2001. 91(1): p. 137-44.

4. Xu, F., et al., Activation of different vestibular subnuclei evokes differential respiratory and pressor responses in the rat. Journal of Physiology, 2002. 544(1): p. 211-223.

5. Bradley, D.J., et al., Cardiovascular and respiratory responses evoked from the posterior cerebellar cortex and fastigial nucleus in the cat. Journal of Physiology, 1987. 393: p. 107-121.

6. Decima, E.E. and C. von Euler, Intercostal and cerebellar influences on efferent phrenic activity in the decerebrate cat. Acta Physiol Scand, 1969. 76(1): p. 148-58.

7. Lutherer, L.O. and J.L. Williams, Stimulating fastigial nucleus pressor region elicits patterned respiratory responses. American Journal of Physiology, 1986. 250: p. R418-R426.

8. Moruzzi, G., Paleocerebellar inhibition of vasomotor and respiratory carotid sinus reflexes. Journal of Neurophysiology, 1940. 3: p. 20-32.

9. Williams, J.L., S.J. Everse, and L.O. Lutherer, Stimulating fastigial nucleus alters central mechanisms regulating phrenic activity. Respiration Physiology, 1989. 76: p. 215-228.

10. Xu, F.D. and D.T. Frazier, Modulation of respiratory motor output by cerebellar deep nuclei in the rat. Journal of Applied Physiology, 2000. 89(3): p. 996-1004.

11. Bianchi, A.L., M. Denavitsaubie, and J. Champagnat, Central control of breathing in mammals: neuronal circuitry, membrane properties, and neurotransmitters. Physiol Rev, 1995. 75(1): p. 1-45.

12. Cohen, M.I., Central determinants of respiratory rhythm. Annual Review of Physiology, 1981. 43: p. 91-104.

13. Duffin, J., K. Ezure, and J. Lipski, Breathing rhythm generation: focus on the rostral ventrolateral medulla. News Physiol Sci, 1995. 10: p. 133-140.

14. Ezure, K., Synaptic connections between medullary respiratory neurons and considerations on the genesis of respiratory rhythm. Progress in Neurobiology, 1990. 35: p. 429-450.

15. Feldman, J.L., Neurophysiology of breathing in mammals, in Handbook of Physiology. The Nervous System, IV. Intrinsic Regulatory Systems of the Brain., F.E. Bloom, Editor. 1986, American Physiological Society: Bethesda, MD. p. 463-524.

16. Miller, A.D., A.L. Bianchi, and B.P. Bishop, Overview of the neural control of respiratory muscles, in Neural Control of the Respiratory Muscles, A.D. Miller, A.L. Bianchi, and B.P. Bishop, Editors. 1997, CRC Press: Boca Raton, FL. p. 1-4.

17. Miller, A.D., et al., Ventral respiratory group bulbospinal inspiratory neurons participate in vestibular-respiratory reflexes. J Neurophysiol, 1995. 73(3): p. 1303-1307.

18. Yates, B.J., M.S. Siniaia, and A.D. Miller, Descending pathways necessary for vestibular influences on sympathetic and inspiratory outflow. Amer J Physiol-Regul Integr C, 1995. 37(6): p. R1381-R1385.

19. Woodring, S.F. and B.J. Yates, Responses of ventral respiratory group neurons of the cat to natural vestibular stimulation. Am. J. Physiol. Regul. Integr. Comp. Physiol., 1997. 273(6): p. R1946-R1956.

20. Yates, B.J., et al., Organization of vestibular inputs to nucleus tractus solitarius and adjacent structures in cat brain stem. American Journal of Physiology, 1994. 267(4): p. R974-R983.

21. Billig, I., et al., Transneuronal tracing of neural pathways controlling an abdominal muscle, rectus abdominis, in the ferret. Brain Research, 1999. 820(1-2): p. 31-44.

22. Billig, I., et al., Definition of neuronal circuitry controlling the activity of phrenic and abdominal motoneurons in the ferret using recombinant strains of pseudorabies virus. J Neurosci, 2000. 20(19): p. 7446-54.

23. Billig, I., et al., Transneuronal tracing of neural pathways controlling abdominal musculature in the ferret. Brain Res, 2001. 912(1): p. 24-32.

24. Shintani, T., et al., Transneuronal tracing of neural pathways influencing both diaphragm and genioglossal muscle activity in the ferret. J Appl Physiol, 2003. 95(4): p. 1453-9.

25. Yates, B.J., et al., Transneuronal tracing of neural pathways controlling activity of diaphragm motoneurons in the ferret. Neuroscience, 1999. 90(4): p. 1501-1513.

26. Peterson, B.W., et al., Responses of medial reticular neurons to stimulation of the vestibular nerve. Experimental Brain Research, 1975. 22: p. 335-350.

27. Peterson, B.W. and C. Abzug, Properties of projections from vestibular nuclei to medial reticular formation in the cat. Journal of Neurophysiology, 1975. 38: p. 1421-1435.

28. Bolton, P.S., et al., Response of pontomedullary reticulospinal neurons to vestibular stimuli in vertical planes. Role in vertical vestibulospinal reflexes of the decerebrate cat. Journal of Neurophysiology, 1992. 67: p. 639-647.

29. Wilkinson, K.A., et al., Responses of feline medial medullary reticular formation neurons with projections to the C5-C6 ventral horn to vestibular stimulation. Brain Research, 2004. in press.

30. Mori, R.L., et al., Role of the medial medullary reticular formation in relaying vestibular signals to the diaphragm and abdominal muscles. Brain Res, 2001. 902(1): p. 82-91.

31. Mateika, J.H. and J. Duffin, The connections from botzinger expiratory neurons to upper cervical inspiratory neurons in the cat. Exp Neurol, 1989. 104(2): p. 138-46.

32. Duffin, J. and R.W. Hoskin, Intracellular recordings from upper cervical inspiratory neurons in the cat. Brain Research, 1987. 435(1-2): p. 351-4.

33. Hoskin, R.W. and J. Duffin, Excitation of upper cervical inspiratory neurons by inspiratory neurons of the nucleus retroambigualis in the cat. Exp Neurol, 1987. 98(2): p. 404-17.

34. Nakazono, Y. and M. Aoki, Excitatory connections between upper cervical inspiratory neurons and phrenic motoneurons in cats. J Appl Physiol, 1994. 77(2): p. 679-683.

35. Tian, G.F. and J. Duffin, Connections from upper cervical inspiratory neurons to phrenic and intercostal motoneurons studied with cross-correlation in the decerebrate rat. Exp Brain Res, 1996. 110(2): p. 196-204.

36. Miller, A.D. and B.J. Yates, Evaluation of role of upper cervical inspiratory neurons in respiration, emesis and cough. Brain Research, 1993. 606: p. 143-147.

37. Bolton, P.S., T. Goto, and V.J. Wilson, Horizontal canal input to upper cervical commissural neurons. Experimental Brain Research, 1993. 92.

38. Endo, K., et al., Response of commissural and other upper cervical ventral horn neurons to vestibular stimuli in vertical planes. J Neurophysiol, 1994. 71(1): p. 11-16.

39. Isu, N., D.B. Thomson, and V.J. Wilson, Vestibulospinal effects on neurons in different regions of the gray matter of the cat upper cervical cord. J Neurophysiol, 1996. 76(4): p. 2439-2446.

40. Schor, R.H., et al., Responses of interneurons in the cat cervical cord to vestibular tilt stimulation. Journal of Neurophysiology, 1986. 56: p. 1147-1156.

41. Thomson, D.B., N. Isu, and V.J. Wilson, Responses of neurons of the cat central cervical nucleus to natural neck and vestibular stimulation. J Neurophysiol, 1996. 76(4): p. 2786-2789.

42. Rossiter, C.D., et al., Changes in outflow to respiratory pump muscles produced by natural vestibular stimulation. J Neurophysiol, 1996. 76(5): p. 3274-3284.

43. Xu, F.D., Z. Zhang, and D.T. Frazier, Transient respiratory augmentation elicited by acute head-down tilt in the anesthetized cat. Journal of Applied Physiology, 1998. 85(2): p. 490-496.

44. Schor, R.H., A.D. Miller, and D.L. Tomko, Responses to head tilt in cat central vestibular neurons. I. Direction of maximum sensitivity. Journal of Neurophysiology, 1984. 51: p. 136-146.

45. Kerman, I.A. and B.J. Yates, Regional and functional differences in the distribution of vestibulosympathetic reflexes. Am. J. Physiol. Regul. Integr. Comp. Physiol., 1998. 275(3): p. R824-R835.

46. Gacek, R.R., The course and central termination of first order neurons supplying vestibular end organs in the cat. Acta-Otolaryngol., 1969. Suppl. 254: p. 1-66.

47. Wilson, V.J., et al., Saccular and utricular input to cat neck motoneurons. Journal of Neurophysiology, 1977. 40: p. 63-73.

48. Abzug, C., et al., Cervical branching of lumbar vestibulospinal axons. With an appendix by C.P. Bean. Journal of Physiology, 1974. 243: p. 499-522.

49. Lipski, J. and J. Duffin, An electrophysiological investigation of propriospinal inspiratory neurons in the upper cervical cord of the cat. Exp Brain Res, 1986. 61(3): p. 625-37.